➔DOI : 10.15740/HAS/AJBS/11.1/162-171
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in*

■ — AFBSAH-2016 — ASIAN JOURNAL OF BIO SCIENCE
Volume **11** | Issue 1 | April, 2016 | 162-171

RESEARCH PAPER

Physiological and molecular characterization of three rice genotypes with differential nitrogen use efficiency

B. SRIKANTH[1], I. SUBHAKARA RAO[1], S. ZEHRA MOIZ[2], K.N. SWAMY[1], K. SUREKHA[1], D. SUBRAHMANYAM[1], L.V. SUBBA RAO[1], P. VIJAYALAKSHMI[1], S.R. VOLETI[1] AND **C.N. NEERAJA[1]**

[1]ICAR- Indian Institute of Rice Research, Rajendranagar, HYDERABAD (TELANGANA) INDIA
[2]Jawaharlal Nehru Technological University, HYDERABAD (TELANGANA) INDIA
Email : cnneeraja@gmail.com

Article Info : Received : 30.12.2015; Accepted : 12.02.2016

Identification and adaptation of rice genotypes with high nitrogen use efficiency (NUE) is a potential approach in optimizing N requirements, lowering the cost of cultivation and reducing the environmental pollution through the reduction of nitrous oxide emission in rice. Three rice genotypes differing in their NUE were grown in hydroponics and field conditions and characterized for physiological, yield parameters and NUE indicators along with expression analysis under low and recommended nitrogen conditions. Significant variations were observed in 18 physiological, yield parameters and 8 NUE indicators among genotypes confirming the genotypic variability for the traits under low nitrogen conditions. Reduction of yield related parameters except thousand grain weight were noted. Correlation analysis of various yield components explains the significance of total biomass for grain yield, straw yield, N content in grain, straw and nitrogen absorption efficiency. Differential expression of *OsNIA2* gene in NUE efficient genotypes *viz*., in shoots of GQ25 and roots of IR55178 in the present study suggests its possible role in N metabolism and is encouraging for exploring the possibilities of improving nitrogen use efficiency in rice.

Key words : NUE, Nitrogen treatment, NUE indicators, ANOVA, Correlation, *OsNIA2* expression

How to cite this paper : Srikanth, B. Rao, I. Subhakara, Moiz, S. Zehra, Swamy, K.N., Surekha, K., Subrahmanyam, D., Rao, L.V. Subba, Vijayalakshmi, P., Voleti, S.R. and Neeraja, C.N. (2016). Physiological and molecular characterization of three rice genotypes with differential nitrogen use efficiency. *Asian J. Bio. Sci.*, **11** (1) : 162-171 [Special Issue of AFBSAH-2016].

INTRODUCTION

Rice is the major staple central crop feeding billions of people around the world. India has ~44 million ha area under rice and is the second largest producer (~105 million tonnes during 2013-14) after China. Rice is not only the staple food of two thirds of Indians but also play a key role in the national food and livelihood security. With the continuous increase in population, rice productivity needs to be enhanced from the present 2.05 t ha^{-1} to 3.3 - 4.05t ha^{-1} in the next 40 years to keep pace with the growing demand for rice and constraints for rice cultivation (*www.drricar.org* (DRR Vision 2050). For complete realization of the higher yield potential and enhanced fertilizer nitrogen (N) application is compulsory (Roberts, 2009) as it is the most important nutrient input for the overall growth and development of plants. Globally about 50 per cent of human population relies on nitrogen fertilizer for food production (Smil, 2001). Urea is the major nitrogen fertilizer and it contributes about 80 per cent to the total fertilizer consumption in India. Globally, it contributes to about 55 per cent of total fertilizer nitrogen consumption. It is estimated that by 2050 about 250 million tonnes of fertilizer N may be needed (Tilman *et al*.,2011). Efficiency of fertilizer nitrogen is quite low, more than 60 per cent of fertilizer N lost to environment through leaching, ammonia volatization, denitrification and surface runoff (Prasad, 2013). Thus, the excess nitrogen consumption increases environmental pollution and also the cost of rice cultivation. With the priority of the low input sustainable and environment friendly agriculture, developing rice genotypes with high NUE becomes one of the major objectives of rice breeding programs in current agricultural scenario. High NUE cultivars can be defined by their ability to produce higher grain yields under low N inputs (Ladha *et al.*, 1998). The efforts for maximum possible yield with optimum inputs of N compensating the compromised yield with economic and environmental benefits are in progress (Srikanth *et al.*, 2015). NUE is likely to be regulated by many different genes with the ability of a plant to remobilize leaf stored N at the grain filling is an important factor for NUE in crops and has been strongly implicated in quantitative trait locus (QTL) studies with cereals (Mickelson *et al.*, 2003). Through gene identification and positional cloning strategies, several genes of N metabolism in rice were characterized and differential expression of several candidate genes directly involved in N metabolism and transcription factors in relation to NUE has been reported in rice under differential N situations (Duan *et al.*, 2007; Fan *et al.*, 2007; Hakeem *et al.*, 2012; Kumar *et al.*, 2003; Li *et al.*, 2006; Qiu *et al.*, 2009; Zhao *et al.*, 2012 and Zhao and Shi, 2006). Of these Nitrate reductase (*OsNIA2*) is one of the key gene for NUE and involves in the first step of nitrate assimilatory pathway, which is a rate-limiting step that regulates the inflow of inorganic nitrogen (NO^{-3}) to organic form in plants. The nitrate taken up by plants is assimilated with the help of enzymes nitrate reductase and nitrite reductase. The end product, NH^{+4}, is incorporated into amino acids via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway (Naik *et al.*, 1982 and Abrol *et al.*, 1988). Under NICRA (National Innovations on Climate Resilient Agriculture) projects, genotypes with differential NUE were identified after screening of hundreds of genotypes under low N condition (*http://www.drricar.org:8000/*). And the present experiment was aimed to study the physiological and yield related parameters along with NUE indicators in three genotypes and also to observe the expression patterns of *OsNIA2* gene in shoot and root samples of these genotypes with differential physiological variability of N efficient and N inefficient genotypes.

RESEARCH METHODOLOGY

Plant material and growth conditions :

Genotypes in this study comprised of one upland variety Somaly 2-023-3-5-1-2-1and two restorer lines, *viz.*, GQ-25 and IR 55178. Rice seeds were surface sterilized in 0.1 per cent $HgCl_2$ solution for 3- 4 min followed by three to four washes with distilled water and germinated in Petridishes with moist blotting paper at 28^0C for 3 days in dark. After germination sprouted seeds were shifted to normal light conditions for 5 days. The eight days old seedlings were shifted to plastic cups containing Yoshida solution (Yoshida *et al.*, 1976) in two treatments *viz.*, with and without N source (NH_4NO_3) and the medium was refreshed for every two days. For the genotypes in both the treatments three biological replications were maintained. The genotypes grown in hydroponics were also cultivated in field conditions under low and recommended nitrogen application during wet season, (*Kharif*) 2013 at the experimental fields of ICAR-Indian Institute of Rice Research, Hyderabad, India for phenotypic study.

Sampling and measurements :

The experiment was performed in Split Plot Design, with and without N application as main plots and genotypes as subplots in three replications. Urea (46.5%) was used as nitrogen fertilizer @ 100 kg ha^{-1} in three equal split applications to the recommended N condition (at basal, maximum tillering and panicle initiation stages). Phosphorus, potassium and zinc(@40, 40 and 25 kg ha^{-1}, respectively), were applied to both plots. The plot size was 15 m^2. To avoid nutrients flow between the plots, the two main plots were divided with polyethylene film by 60 cm underground. Rice seedlings grown in hydroponics were transplanted in the two plots with a spacing of 20 x 15 cm. From each plot, six representative hills were harvested at maturity and were divided into vegetative and reproductive parts, dried and weighed for determining dry matter of various plant parts. Grain and straw yield was expressed in t ha^{-1} and adjusted to 14 per cent grain moisture content. Straw and grain samples were analyzed for nitrogen with Kjeldahl method. Plant height, number of tillers, panicle bearing tillers, number of panicles per hill, filled and unfilled grain weight, grain yield (t ha^{-1}), thousand grain weight (g), straw yield (t ha^{-1}), N per cent in grains, N per cent in straw and total N per cent in plant and also harvest index were recorded. NUE indicators *viz.*, agronomic efficiency (AE), physiological efficiency (PE), agro physiological efficiency (APE), apparent recovery efficiency (ARE), utilization efficiency (UE), grain yield efficiency index (GYEI), N grain production efficiency (NGPE) and N absorption efficiency (NAE) were calculated using grain yield and total N uptake as earlier reported by Fageria and Baligar (2003).

Statistical analysis of data :

The numerical data were subjected to statistical analysis using Excel 2007. Correlations among all the parameters of three genotypes were calculated. The physiological data were analyzed as factorial design and two way analysis of variance (ANOVA) was performed using an open source software R (Team, 2012) with agricolae package (Felipe de Mendiburu, 2012).

RNA isolation, c-DNA synthesis and qRT-PCR analysis :

Hydroponically grown three genotypes shoot and root samples were collected from low and recommended N condition and immediately frozen in liquid nitrogen for RNA isolation. Total RNA was isolated using TRizol reagent (Invitrogen, USA) and the RNA was quantified using Nanodrop® ND1000 spectrophotometer (Thermo Scientific, USA). To remove DNA, RNA samples were treated with RNAse free DNAse (Invitrogen, USA). Approximately 1 µg of total RNA from each sample was used as template for the first-strand cDNA synthesis, using Superscript III reverse transcriptase (Invitrogen, USA). Gene-specific primers (forward primer: GGAG GACGGGTGGGAGTA, reverse primer: TTCAGAAG ACGAGGCAGGAC) for *OsNIA2* gene were selected from the previous report of Fan *et al.* (2007). qRT-PCR was performed using Applied Biosystems 7500 Real Time PCR (Life Technologies, USA), in a final volume of 20µl, containing 10µl of Platinum® SYBR® Green qPCR Super Mix (Invitrogen, USA) with 500 nM each of forward and reverse primers and 20 ng of cDNA samples. The real time PCR cycling conditions included a pre-incubation at 50°C for 2 min and denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. qRT-PCR was performed in three biological replicates. Samples were run in duplicates on the same plate along with controls set up for each sample in duplicate using 18s RNA gene (forward primer: CTACGTCCCTGCC CTTTGTAC, reverse primer: ACACTTCACCGG ACCATTCAA) was used to normalize gene expressions. The data were analyzed using the 7500 Sequence Detection Software (Applied Biosystems, USA). The fold change expression of gene was calculated using the $2^{-\Delta CT \Delta CT}$ method, which represents the difference of CT between the control products and the target gene products. Recommended N condition was taken as control and low N was considered as treated.

RESEARCH FINDINGS AND ANALYSIS

Significant variation was observed for 18 physiological and yield related parameters along with eight NUE indicators in three genotypes identified for their differential NUE studied under low and recommended N condition with a familiar trend of decrease of all the physiological and yield related parameters under low N condition (Table 1), as it is known that N plays key role in total biomass and there by yield (Hirel *et al*., 2007). Analysis of variance (ANOVA) of the data in the present study showed significant differences among the genotypes for 17 parameters (Table 1) but for unfilled grain weight. The interaction between treatment and entry has shown significant variation for 17 parameters indicating that genotypes differing in response to N condition. Several earlier reports also showed significant variation among genotypes in terms of physiological and yield parameters under low N condition suggesting the existence of genotypic variability. (Gueye and Becker 2011; Kanfany *et al.*, 2014 and Mahajan *et al.*, 2012). Significant variation among genotypes was observed in NUE indicators such as, agronomic efficiency (AE), physiological efficiency, agro physiological efficiency, apparent recovery efficiency per cent, utilization efficiency, internal NUE, absorption NUE, grain yield efficiency index, N grain production efficiency and N absorption efficiency (NAE) indicating the role of grain yield in NUE indicators.

In the present study plant height was reduced by 21 per cent under low N conditions (Table 1) as reported in the earlier studies (Gueye and Becker, 2011; Kanfany *et al.*, 2014; Mahajan *et al.*, 2012; Ntanos and Koutroubas, 2002 and Zhao *et al.*, 2012). Number of tillers per hill (Table 1) reduced by 24.4 per cent under low N condition. Similar results of decrease in tiller number were observed in earlier reports (Gueye and Becker, 2011; Singh *et al.*, 1998 and Srikanth *et al.*, 2015). Nitrogen application is associated with promotion of tillering in rice with the significant different number of tillers between basal and additional N fertilization as well as the top dressing (Lee *et al*., 2010 and Wells and Faw, 1978). Panicle bearing tillers per hill were reduced by 36.87 per cent, number of panicles per hill were reduced by 35.63 per cent and panicles weight per hill reduced by 47.72 per cent (Table 1) under low N condition in comparison with recommended N were noted in the present study. Thus, the panicle number is apparently correlated with tiller number, however, the increase in number of tillers do not

Table 1: Analysis of variance (ANOVA) of yield components, nitrogen content and NUE indicators along with expression of *OsNIA2* gene in three genotypes under low and recommended nitrogen conditions

	Range		Mean					
	Low N	Rec N	Low N	Rec N	Treatment (T)	Entry (E)	T x E	CV%
Plant height (cm)	65.33- 119.66	95.66- 157.33	96.66	121.66	**	**	**	5.55
Number of tillers	3.33- 11.33	5.66- 21	8.22	10.88	*	**	**	19.07
Panicle bearing tillers (no)	3.33- 8.33	5.33-21	6.66	10.55	**	**	**	17.44
Panicle number/ hill	3.66- 6.33	6- 11.66	5.22	8.11	**	**	**	15
Panicle weight/ hill (g)	4.68- 20.25	8.10- 28.40	10.47	20.03	**	**	**	7.07
Filled grain weight/ hill (g)	3.74- 15.90	7.61- 23.77	8.44	17.33	**	**	**	2.11
Unfilled grain weight/ hill (g)	0.71- 1.66	0.48- 2.48	1.24	1.31	NS	NS	NS	24.06
Straw weight/ hill (g)	6.98- 19.51	6.81- 40.89	12.73	19.95	**	**	**	3.21
Total grain weight/ hill (g)	4.46- 17.56	8.07- 26.25	9.68	18.64	**	**	**	7.36
Thousand grain weight	13.18- 29.52	15.65- 25.55	18.83	19.03	**	**	**	1.32
Grain yield (t ha^{-1})	2.23- 8.78	4.04- 13.12	4.84	9.32	**	**	**	7.36
Straw yield (t ha^{-1})	3.50- 9.75	3.41- 20.44	6.36	9.97	**	**	**	3.21
N% in grain	0.99- 1.15	1.07-1.39	1.06	1.27	**	**	**	2.42
N% in straw	0.32- 0.37	0.38- 0.46	0.34	0.43	**	**	*	3.24
N% in plant	1.31- 1.49	1.45- 1.85	1.41	1.7	**	**	**	2.12
Spikelet fertility %	59.69- 87.42	63.59- 88.94	71.27	72.49	**	**	**	0.93
Total biomass (t ha^{-1})	7- 18.53	10.11- 31.24	11.21	19.3	**	**	**	4.22
Harvest index	24.42- 44.71	33.39- 73.75	37.52	47.48	**	**	**	5.76
NGPE (kg/kg)	54.2- 70.16	47.77- 67.35	63.16	56.95	**	**	**	3.92
NAE%	20.48- 67.43	40.77- 92.88	37.63	75.11	**	NS	**	7.13
AE (kg kg^{-1})		41.16- 47.75		44.78		**		11.89
PE (kg kg^{-1})		121- 123.40		122.28		**		5.59
APE (kg kg^{-1})		67.05- 80.68		72.93		**		11.57
ARE (%)		80.37- 84.54		82.46		**		13.02
UE (kg kg^{-1})		94.04- 107.82		102.65		**		10.66
GYEI		0.71- 0.82		0.78		*		19.95
OsNIA2 shoot	0.37- 0.52		0.47			**		11.28
OsNIA2 root	1.41- 2.37		1.74			*		15.66

Data indicate mean of 3 genotypes. * and ** indicate significance of values at P=0.05 and 0.01, respectively; NS= Non-significant by ANOVA. NGPE denotes for N grain production efficiency, NAE denotes for Nitrogen absorption efficiency, AE denotes for Agronomic efficiency, PE denotes for Physiological efficiency, APE denotes for Agro physiological efficiency, ARE denotes for Apparent recovery efficiency, UE denotes for Utilization efficiency, GYEI denotes for Grain yield efficiency index. For real time expression recommended N condition taken as control and low N was considered as treated always result in the increase in panicle number. Similar results of significant increase in panicle bearing tillers, panicle number and panicle weight in increase in N application were reported (Artacho *et al.*, 2009; Geoffrey *et al.*, 2012; Metwally *et al.*, 2010; Sui *et al.*, 2013; Srikanth *et al.*, 2015 and Zhang *et al.*, 2013).

Total grain weight per hill was reduced by 48 per cent, filled grain weight per hill reduced by 51.3 per cent and unfilled grain weight per hill reduced by 5.34 per cent (Table 1) under low N condition in comparison with recommended N condition was observed in the present study. Similar results of decrease in total grain weight, filled and unfilled grain weight were reported in studies (Koutroubas and Ntanos, 2003 and Zhang *et al.*, 2013). Spikelet fertility per cent was reduced by 1.7 per cent under low N condition in comparison with recommended N application, as earlier studies of Singh *et al.* (1998) also observed the reduction in spikelet fertility per cent. Thousand grain weight was reduced by about 1 per cent under low N conditions was observed in the present study, grain weight being a stable varietal character with spikelet size controlled by hull size (Gueye and Becker, 2011; Hasegawa, 2003; Singh *et al.*, 1998; Yoshinaga *et al.*, 2013 and Zhang *et al.*, 2013).

Grain yield was reduced by 48 per cent under low N condition in the present study (Table 1). Grain yield reported to be associated with number of productive tillers, panicles and spikelets, thus, the reduction in number of productive tillers, panicles and spikelets under low N condition could have reduced the yield drastically (Artacho *et al.*, 2009; Kanfany *et al.*, 2014; Mae *et al.*, 2006; Sui *et al.*, 2013 and Tirol-Padre *et al.*, 1996). About 36 per cent reduction in straw yield was observed in the present study. It is reported that N application could contribute to grain yield more by enhancing the total dry matter, thus, under low nitrogen condition reduction in dry matter accumulation and subsequent decrease in grain yield. (De Datta *et al.*, 1990; Gueye and Becker, 2011 and Singh *et al.*, 1998).

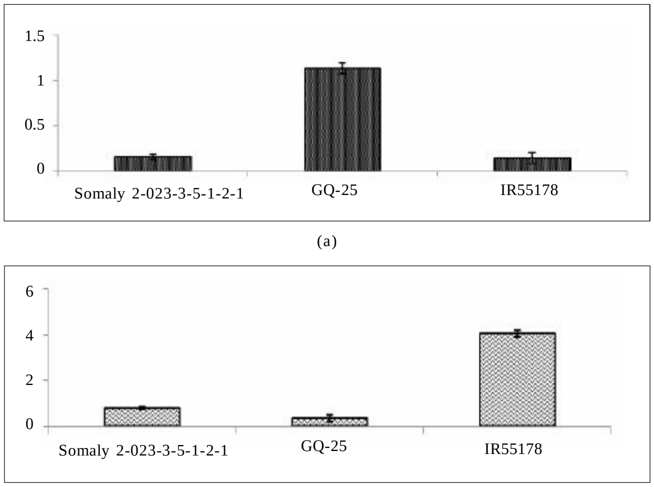
In the present study about 16.53 per cent reduction of N per cent in grains, 20.93 per cent reduction of N per cent in straw and overall 17.05 per cent reduction of total N per cent (Table 1) in plant was observed under low N condition in comparison with recommended N condition. There were reports that N content in grain, straw and their association to biomass and yield were highly variable across the locations, genotypes, stage of N application and sampling (Artacho *et al.*, 2009; De Datta *et al.*, 1990; Gueye and Becker, 2011; Singh *et al.*, 1998; Yoshinaga *et al.*, 2013 and Zhang *et al.*, 2009). About 42 per cent reduction in the mean total biomass and 21 per cent reduction in harvest index (Table 1) were noted in three genotypes grown under low N condition in comparison with recommended N condition. Earlier reports also showed decrease in biomass and harvest index under low N condition (Artacho *et al.*, 2009; De Datta *et al.*, 1990; Inthapanya *et al.*, 2000; Mae *et al.*, 2006 and Singh *et al.*, 1998).

In the present study (Table 1) agronomic efficiency (difference of grain yield between recommended and low N/ N applied) of genotypes ranged from 41.16 to 47.75 kg kg^{-1} with a mean of 44.8 kg kg^{-1} not within the reported range of agronomic efficiency as 15 to 25 kg grain produced per kg N applied in low land rice in tropics (Yoshida *et al.*, 1981) and 20 kg grain per kg N in long duration varieties as explained by Singh *et al.* (1998). Physiological efficiency (difference of biological yield between recommended and low N/ difference of N content between recommended and low N) of genotypes ranged from121 to 123.40 kg kg^{-1} (mean 122.28 kg kg^{-1}) and the values of physiological efficiency are similar as reported by Fageria and Baliger (2003). Agro physiological efficiency (difference of grain yield between recommended and low N/ difference of N content between recommended and low N) of genotypes was slightly higher (72.93 kg kg^{-1}) than the earlier report of Singh *et al.* (1998), observed 64 kg grain per kg of N uptake.Apparent recovery efficiency per cent (difference between total N content of recommended and low N/ N applied x 100) also showed higher (82.46%) than the reported mean ARE of 39 per cent (Fageria and Baliger (2003) and mean ARE of 43 per cent in medium duration varieties (Singh *et al.*, 1998). Surprisingly mean utilization efficiency of genotypes was high and 102.65 kg of yield per kg N applied. Our results substantiated the earlier report under Brazilian conditions are in the range of 36 to 83 and average across the N rates was 58 kg kg^{-1} reported by Fageria and Baliger (2003). These differences in efficiencies might be due to the genotypic variation in the cultivars. Mean N grain production efficiency was increased by 9.8 per cent under low N condition (63.16) in comparison with recommended N condition (56.95). This was slightly higher than earlier report, 54.3 in HPNUE genotypes reported by Singh *et al.* (1998). There is reduction in N absorption efficiency about 50 per cent was noted in the low N in comparison with recommended

N condition. It was reported that NAE in various rice genotypes first increased, peaked under a medium nitrogen rate and then decreased, in a pot experiment (Feng *et al.*, 2011). Grain yield is the best measure of genotype evaluation in screening. Screening results can be interpreted using grain yield efficiency index (GYEI= (yield at low nutrient level) x (yield at high nutrient level)/ (experimental mean at low nutrient level) x (experimental mean at high nutrient level) as explained by Fageria *et al.* (1988). In the present study mean GYEI of genotypes recorded is 0.78. Metwally *et al.* (2010) explained in the earlier report that there was a wide variation in GYEI among genotypes under low and high nitrogen condition.

Expression analysis of *OsNIA2* gene under low N situations :

Using qRT-PCR the expression levels of *OsNIA2* gene in shoot and root samples of three genotypes (Fig. 1 and 2) were studied under low N conditions here recommended N condition was taken as control. Expression of *OsNIA2* gene was down regulated by 84.72 per cent in shoot samples, 18.47 per cent in root samples of Somaly 2-023-3-5-1-2-1, 86 per cent decrease in shoot samples of IR55178, 64.48 per cent decrease in root samples of GQ-25 under low N condition in comparison with recommended N condition was noted (Fig. 1 and 2). Decreased expression of *OsNIA2* gene in shoot samples after N starvation was observed in nitrogen sensitive cultivar but not changed in N use efficient cultivar (Fan *et al.*, 2007). Expression of *OsNIA2* gene was up regulated by 75.30 per cent in shoot samples of IR55178, 11.13 per cent in shoot samples of GQ-25 under low N condition (Fig. 1a and b) in comparison with recommended N condition was noted. Enhanced expression of *OsAMT1;1* and *OsNRT2;1* under low N condition have also been demonstrated in rice seedlings hydroponics (Shi *et al.*, 2010). Differential expression of nitrate reductase and glutamine synthetase between low and high nitrogen use efficient cultivars indicated the enzymes to be involved in efficient uptake and utilization (Hakeem *et al.*, 2012). Enhanced expression of *OsSPL14* gene in leaf and three panicle stages reported to be associated with yield components were observed under low nitrogen condition was reported by Srikanth *et al.* (2015). These differences in expression of *OsNIA2* gene reflect the distinction between the cultivars and found to be associated with differential expression. Though the enhanced expression of *OsNIA2* in shoots of GQ-25 and roots of IR 55178 were observed, the samples were collected at single time point. Studies on temporal expression of the gene across tissues might converge and clarify the role of *OsNIA2* in NUE efficient genotype.

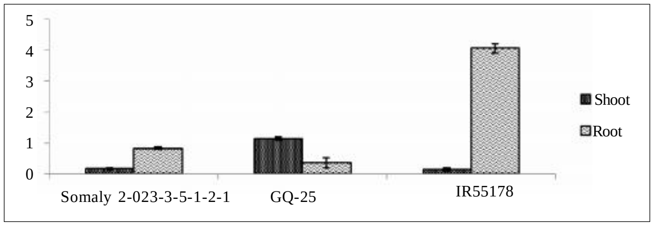


(a)

(b)

Here recommended N condition was used as control and low N as treated

Fig. 1: (a and b): Differential expression of *OsNIA2* in shoot (a) and root (2) samples of Somaly 2-023-3-5-1-2-1, GQ-25 and IR55178 under low N condition



Here recommended N condition was used as control and low N as treated

Fig. 2 : Differential expression of *OsNIA2* gene in shoot and root samples of Somaly 2-023-3-5-1-2-1, GQ-25 and IR55178 under low N condition.

Correlation studies :

Under low nitrogen conditions (Table 2) number of tillers per hill significantly (P<0.05) correlated with panicle bearing tillers per hill. Total grain weight per hill have shown significant correlation with (P<0.05) thousand grain weight, (P<0.01) total filled grain weight per hill, total N content in plant, N absorption efficiency, (P<0.001) N content in grain and grain yield. Total panicles weight per hill have shown significant correlation with (P<0.05) total grain weight per hill, thousand grain weight, grain yield, (P<0.01) total filled grain weight per hill, N content in grain, total N content in plant and N absorption efficiency. Filled grain weight per hill significantly correlated with ($P<0.05$) thousand grain weight and ($P<0.01$) grain yield. Thousand grain weight have shown significant correlation with ($P<0.01$) grain yield. Straw yield have shown correlation with ($P<0.05$) N content in straw. Earlier report of Zhao *et al.* (2012) suggests that there was a significant correlation between grain yield and straw weight under non-basal N fertilizer application. Total biomass (Table 2) have shown significant correlation with ($P<0.05$) total grain weight per hill, total panicles weight per hill, filled grain weight per hill, N content in grain, grain yield, straw yield, total N content in plant, N content in straw and N absorption efficiency. Ntanos and Koutroubas (2002) reported that in rice grain yield was significantly correlated with dry matter translocation efficiency and nitrogen translocation efficiency suggesting the role of the sink capacity. N content in grain (Table 2) correlated with ($P<0.05$) thousand grain weight, ($P<0.01$) total filled grain weight per hill, total N content in plant and ($P<0.001$) grain yield. Total N content in plant have shown significant correlation with ($P<0.05$) thousand grain weight, ($P<0.01$) total filled grain weight per hill and grain yield. Nitrogen absorption efficiency (Table 2) was significantly correlated with ($P<0.05$) thousand grain weight, ($P<0.01$) total filled grain weight per hill, N content in grain, grain yield and ($P<0.001$) Total N content in plant. Nitrogen is significantly correlated with grain yield, panicle mass, panicle density, grain mass and N translocation ratio (Samonte *et al.*, 2006). Thus, the correlations of yield and NUE parameters appear to be variable as in relation to the earlier studies of correlations of yield parameters suggesting that correlations change considerably with nitrogen treatment and varieties (Srikanth *et al.*, 2015; Sui *et al.*, 2013 and Zhang *et al.*, 2013).

Conclusion :

The three rice genotypes identified for differential NUE, chosen for this study and characterized for 18 physiological, yield related parameters along with eight NUE indicators and expression of *OsNIA2* in shoot and root samples under low and recommended nitrogen conditions. Significant variations were observed in physiological and yield parameters under low N conditions among genotypes confirming the existence of genotypic variation for the traits. There is a reduction in almost all the yield related parameters (Table 1) except thousand grain weight. Correlation analysis (Table 2) of various yield parameters showed the significance of total biomass for grain yield, straw yield, N per cent in grain, straw and nitrogen absorption efficiency. Though NUE is a complex trait and cannot be explained by a single gene, however, differential expression (Fig. 1 and 2) of *OsNIA2* gene in three genotypes *viz.*, Somaly 2-023-3-5-1-2-1, GQ-25 and IR 55178 under low nitrogen condition in the present study suggests its possible role in N metabolism and the study as well encouraging for exploring the possibilities of improving nitrogen use efficiency in rice.

Table 2: Correlation of yield components, nitrogen content and NUE indicators in low N conditions

	Panicle bearing tillers	Grain weight/ hill (g)	Panicle weight/ hill (g)	Thousand grain weight (g)	Filled grain weight/ hill (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	N% in Grain	N% in Straw	N% in plant	NAE%
Number of tillers/ hill	0.961*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Grain weight/ hill (g)	NS	NS	NS	0.977*	0.994**	1***	NS	0.999***	NS	0.994**	0.994**
Panicle weight/ hill (g)	NS	0.986*	NS	0.962*	0.996**	0.986*	NS	0.992**	NS	0.994**	0.994**
Filled grain weight/ hill (g)	NS	NS	NS	0.972*	NS	0.994**	NS	NS	NS	NS	NS
Thousand grain weight (g)	NS	NS	NS	NS	NS	0.976*	NS	NS	NS	NS	NS
Straw yield (t ha^{-1})	NS	NS	NS	NS	NS	NS	NS	NS	0.985*	NS	NS
Total biomass (t ha^{-1})	NS	0.962*	0.977*	NS	0.970*	0.962*	0.954*	0.965*	0.976*	0.985*	0.985*
N% in grain	NS	NS	NS	0.977*	0.995**	0.999***	NS	NS	NS	0.995**	NS
N% in plant	NS	NS	NS	0.956*	0.995**	0.994**	NS	0.951*	NS	NS	NS
NAE%	NS	NS	NS	0.956*	0.995**	0.994**	NS	0.995**	NS	1***	NS

Only significant correlations were shown. *, ** and *** indicate significance of values at P=0.05, 0.01 and 0.1, respectively; NAE denotes for Nitrogen absorption efficiency; NS= Non-significant

Acknowledgment :

This work was supported by the National Initiative on Climate Resilient Agriculture (NICRA), Indian Council of Agricultural Research (ICAR), Ministry of Agriculture, Govt. of India [F. No. Phy/NICRA/ 2011- 2012].

LITERATURE CITED

Abrol, Y.P., Dhar, D. and Kumar, P.A. (1988). Nitrogen metabolism. In: *Plant physiological research in India* (SP Sen Ed.) Published by the Society for *Plant Physiol. & Biochem.*, 75–83pp.

Artacho, P., Bonomelli, C. and Meza, F. (2009). Nitrogen application in irrigated rice growth in Mediterranean conditions: effects on grain yield, dry matter production, nitrogen uptake, and nitrogen use efficiency. *J. Plant Nutr*., **32** : 1574–1593.

De Datta, S.K., Buresh, R.J. and Mamaril, C.P. (1990). Increasing nutrient use efficiency in rice with changing needs. *Fert. Res*., **26** : 157–167.

Duan,Y.H., Zhang, Y.L., YE, L.T., FAN, X.R., XU, G.H. and Shen, Q.R. (2007). Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. *Ann. Bot*., **99** (6) :1153-1160.

Fageria, N.K. and Baligar, V.C. (2003). Fertility management of tropical acid soils for sustainable crop production. In: Rengel, Z. (Ed.), *Handbook of soil acidity*, pp. 359–385.

Fageria, N.K., Morais, O.P., Baligar, V.C. and Wright, R.L. (1988). Response of rice cultivars to phosphorus supply on an oxisol. *Ferti. Res*., **16** : 195– 206.

Fan, X., Jia, L. and Li, Y. (2007). Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. *J. Exp. Bot*., **58** : 1729–1740.

Feng, C.J., Yan, J.H., Bai, L.Y., Bo, D.T. and Xing, C.W. (2011). Methods on identification and screening of rice genotypes with high nitrogen efficiency. *Rice Sci.*, **18** (2) : 127-135.

Geoffrey, O., Asea, G., Lamo, J. and Kikafunda, J. (2012). Comparison of response to nitrogen between upland NERICAs and ITA (*Oryza sativa*) rice varieties. *Science*, **4** : 197–205.

Gueye, T. and Becker, H. (2011). Genetic variation in nitrogen efficiency among cultivars of irrigated rice in Senegal. *J. Agric. Biotech. Sustain. Dev*., **3** : 35–43.

Hakeem, K.R., Chandna, R., Ahmad, A. and Iqbal, M. (2012). Physiological and molecular analysis of applied nitrogen in rice genotypes. *Rice Sci*., **19** : 213–222.

Hasegawa, H. (2003). Crop ecology, management and quality-high yielding rice cultivars perform best even at reduced nitrogen fertilizer rate. *Crop Sci*., **43** : 921–926.

Hirel, B.,Gouis, J.L. and Ney, B. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot*., **58** : 2369–2387.

Inthapanya, P., Sipaseuth, S.P., Sihathep, V., Chanphengsay, M., Fukai, S. and Basnayake, J. (2000). Genotype differences in nutrient uptake and utilisation for grain yield production of rainfed lowland rice under fertilized and non-fertilized conditions. *Field Crop Res*., **65** : 57–68.

Kanfany, G., Namaky, R.E. and Ndiaye, K. (2014). Assessment of rice inbred lines and hybrids under low fertilizer levels in Senegal. *Sustainability*, **6** : 1153–1162.

Koutroubas, S.D. and Ntanos, D.A. (2003). Genotypic differences for grain yield and nitrogen utilization in Indica and Japonica rice under Mediterranean conditions. *Field Crop Res.*, **83** : 251–260.

Kumar, A., Silim, S.N., Okamoto, M., Siddiqi, M.Y. and Glass, A.D.M. (2003). Differential expression of three members of the AMT1 gene family encoding putative high-affinity NH4+ transporters in roots of *Oryza sativa* subspecies indica. *Plant Cell Environ*., **26** : 907–914.

Ladha, J.K., Tirol-Padre, A., Punzalan, G.C., Castillo, E., Singh, U. and Reddy, C.K. (1998). Nondestructive estimation of shoot nitrogen in different rice genotypes. *Agron. J.*, **90**: 33- 40.

Lee, J.H., Kang, C.S., Roh, A.S., Park, K.Y. and Lee, H.J. (2010). Assessment of N top dressing rate at panicle initiation stage with chlorophyll meter based diagnosis in rice. *J. Crop. Sci. Biotechnol.,* **12** : 195–200.

Li, Q., Chen, F. and Sun, L. (2006). Expression profiling of rice genes in early defense responses to blast and bacterial blight pathogens using cDNA microarray. *Physiol. Mol. Plant Pathol.,* **68** : 51–60.

Mae, T., Inaba, A., Kaneta, Y., Masaki, S., Sasaki, M., Aizawa, M., Okawa, S., Hasegawa, S. and Makino, A. (2006). A large-grain rice cultivar, Akita-63, exhibits high yields with high physiological N-use efficiency. *Field Crop Res*., **97** : 227–237.

Mahajan, G., Timsina, J., Jhanji, S., Sekhon, N.K. and Singh, K. (2012). Cultivar response, drymatter partitioning, and nitrogen use efficiency in dry direct-seeded rice in northwest India. *J. Crop Improv.,* **26** : 767–790.

Metwally, T.F., Sedeek, S.E.M., Abdelkhalik, A.F., El-Rewinyl, I.M. and Metwali, E.M.R. (2010). Genetic behaviour of some rice (*Oryza sativa* L.) genotypes under different treatments of nitrogen levels. *Ele. J. Plant Breed.*, **1** : 1266–1278.

Mickelson, S., See, D. and Meyer, F.D. (2003). Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. *J. Exp. Bot.,* **54** : 801-812.

Naik, M.S., Abrol, Y.P., Ramarao, C.S. and Nair, T.V.R. (1982). Nitrate assimilation its regulation and relationship to reduced nitrogen in higher plants. *Phytochemistry,* **21 :** 495–504.

Ntanos, D.A. and Koutroubas, S.D. (2002). Drymatter and N accumulation and translocation for Indica and Japonica rice under Mediterranean conditions. *Field Crop Res*., **74** : 93–101.

Prasad, R. (2013). Fertilizer nitrogen, food security, health and the environment, *Proc. Indian Natn Sci Acad* **79** 4 : December 2013, Spl. Issue, Part B, pp. 997-1010.

Qiu, X., Xie, E.W. and Lian, E.X. (2009). Molecular analyses of the rice glutamate dehydrogenase gene family and their response to nitrogen and phosphorous deprivation. *Plant Cell Rep*.,**28** :1115–1126.

Roberts, T. (2009). The role of fertilizer in growing the world's Food. *Better Crops,* **93**(2): 12-15.

Samonte, S.O.P.B., Wilson, L.T., Medley, J.C., Pinson, S.R.M., McClung, A.M. and Lales, J.S. (2006). Nitrogen utilization efficiency: relationships with grain yield, grain protein, and yield-related traits in rice. *Agron. J.,* **98** : 168–176.

Shi, W.M., Xu, W.F., Li, S.M., Zhao, X.Q. and Dong, G.Q.(2010). Responses of two rice cultivars differing in seedling stage nitrogen use efficiency to growth under low-nitrogen conditions. *Plant Soil,* **326** (1) : 291-302.

Singh, U., Ladha, J.K., Castillo, E.G., Punzalan, G.C., Tirol-Padre, A. and Duqueza, M. (1998). Genotypic variation in nitrogen use efficiency in medium- and long-duration rice. *Field Crop Res*., **58** : 35–53.

Smil, V. (2001). Nitrogen in crop production. An account of global flows. *Glob Biogeochem. Cycles,* **13**:647–662.

Sui, B., Feng, X., Tian, G., Hu, X., Shen, Q. and Guo, S. (2013). Optimizing nitrogen supply increases rice yield and nitrogen use efficiency by regulating yield formation factors. *Field Crop Res*., **150** : 99–107.

Tilman, D., Balzer, C., Hill, J. and Befort, B.L. (2011). Global food demand and the sustainable intensification of agriculture. *Proc. Nat. Acad. Sci.,* **108**(50): 20260-20264.

Tirol-Padre, A., Ladha, J.K., Singh, U., Laureles, E., Punzalan, G. and Akita, S. (1996). Grain yield performance of rice genotypes at sub-optimal levels of soil N as affected by N uptakes and utilization efficiency. *Field Crop Res*., **46** : 127–142.

Wells, B.R. and Faw, W.F. (1978). Short-statured rice response to seeding and N rate. *Agron.J.,* **70** : 477–480.

Yoshida, S., Forno, D.A., Cock, J.H. and Gomez, K.A. (1976). *Laboratory manual for physiological studies of rice*. 3rd Ed., International Rice Research Institute (IRRI), Los Banos, Philippines. pp. 14-16.

Yoshida, S., Satake, T. and Mackill, D.S. (1981). High temperature stress in rice. *Internat. Rice Res. Institute Res. ,* **67** : 1–15.

Yoshinaga, S., Takaib, T., Arai-Sanohb, Y., Ishimaruc, T. and Kondob, M. (2013). Varietal differences in sink production and grain-filling ability in recently developed high yielding rice (*Oryza sativa* L.) varieties in Japan. *Field Crop Res.* **150** : 74–82.

Zhang, L., Lin, S., Bouman, B.A.M., Xue, C., Wei, F., Tao, H., Yang, X., Wang, H., Zhao, D. and Dittert, K. (2009). Response of aerobic rice growth and grain yield to N fertilizer at two contrasting sites near Beijing, China. *Field Crop Res*.,**114**:45-53.

Zhang, W.F., Dou, Z.X., He, P., Ju, X.T., Powlson, D., Chadwick, D., Norse, D., Lu, Y.L., Zhang, Y., Wu, L., Chen, X.P., Cassman, K.G. and Zhang, F.S. (2013). New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proc. Nat. Acad. Sci. U. S. A.*, **110** : 8375–8380.

Zhao, S., Zhao, X. and Shi, W. (2012). Genotype variation in grain yield response to basal N fertilizer supply among different rice cultivars. *J. Biotechnol.*, **11** : 12298–12304.

Zhao, X.Q. and Shi, W.M. (2006). Expression analysis of the glutamine synthetase and glutamate synthase gene families in young rice (*Oryza sativa*) seedlings. *Plant Sci*., **170** : 748–754.

WEBLIOGRAPHY

Mendiburu, F.D. (2012). Agricolae: statistical procedures for agricultural research. R package version 1.1–2. *http://CRAN.R-project.org/package=agricolae.*

Srikanth, B., Subhakara Rao, I., Surekha, K., Subrahmanyam, D., Voleti, S.R. and Neeraja, C.N. (2015). Enhanced expression of OsSPL14 gene and its association with yield components in rice (*Oryza sativa*) under low nitrogen conditions. Gene.*http://dx.doi.org/10.1016/j.gene.2015.10.062.*

Team, R. Core (2012). R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (ISBN 3-900051-07-0). (*http://www.R-project.org*).

AFBSAH-2016 ★★★★★ International Conference on ★★★★★ JSBB, SHIATS

ADVANCING FRONTIERS IN BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE AND HEALTH

11th **Year**
★★★★★ **of Excellence** ★★★★★